

# Comparative Water Relations of *Phaseolus vulgaris* L. and *Phaseolus acutifolius* Gray<sup>1</sup>

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## ABSTRACT

Leaf area expansion, dry weight, and water relations of *Phaseolus vulgaris* L. and *P. acutifolius* Gray were compared during a drying cycle in the greenhouse to understand the characteristics which contribute to the superior drought tolerance of *P. acutifolius*. Stomates of *P. acutifolius* closed at a much higher water potential than those of *P. vulgaris*, delaying dehydration of leaf tissue. *P. acutifolius* had a more deeply penetrating root system, which also contributes to its drought tolerance. Root-shoot ratios did not differ between the two species either under well watered or water stressed conditions. Leaf osmotic potential was also similar in the two species, with no apparent osmotic adjustment during water stress. These results indicate that *P. acutifolius* postpones dehydration and suggest that sensitive stomates and a deeply penetrating root system are characteristics which, if incorporated into cultivated beans, might increase their drought tolerance.

*Phaseolus vulgaris* L., the common bean, provides an important source of protein in many developing countries. However, it is extremely sensitive to drought, and significant yield reductions due to mild drought are common (4, 12). A related species, *Phaseolus acutifolius* Gray, tepary bean, has been reported to produce extremely well with limited moisture (2, 3, 13, 15, 20, 21).

The advantage of using exotic bean germ plasm to improve the tolerance of cultivated beans to environmental stress has been advocated by several authors (16, 19). To realize this advantage, an understanding of the qualities which contribute to increased tolerance is necessary. Despite the demonstrated ability of tepary bean to produce high yields under dry land agriculture, there is a paucity of information on the features which result in its drought tolerance.

Two general methods by which plants cope with a dry environment have been described (9). A dehydration tolerator can withstand appreciable loss of water and still photosynthesize and grow at low water potentials. A dehydration postponer cannot withstand low tissue water potentials, but has characteristics which prevent or postpone the loss of tissue water and maintain high leaf water potentials and turgor. Characterizing mechanisms by which tepary bean copes with a dry environment will better enable researchers to plan breeding programs to move desirable qualities into cultivated common beans.

Experiments described in this communication were designed

to compare *P. vulgaris* and *P. acutifolius* for growth and water relations responses during a drying cycle to determine the mechanisms by which *P. acutifolius* withstands drought conditions.

## MATERIALS AND METHODS

*Phaseolus vulgaris* L. var Pinto and var WHR,<sup>2</sup> and *P. acutifolius* Gray accession MN 258/78, were grown in 8-inch pots in a 1:1 volume mix of sand and turface<sup>3</sup> in a "temperature controlled" greenhouse at the University of Minnesota, St. Paul, MN. Experiments were conducted during the summers of 1982 and 1983 under natural photoperiod, with day temperatures between 25° and 35°C and night temperatures 20° to 23°C.

Seeds were planted 3 per pot and seedlings thinned to 1 per pot 3 d after emergence. Soil was watered twice daily to the drip point with alternate flushes of distilled H<sub>2</sub>O and 0.5 modified Hoagland solution. After 2 weeks of growth, water was withheld from one set of plants until the *P. vulgaris* plants remained wilted through the night. This usually took 6 to 8 d. Plants were then watered with 0.5 modified Hoagland solution. Leaf area was measured nondestructively every 1 to 2 d from 14 DAP to termination of the experiment. Midday, between 1 and 3 PM, leaf water potential, leaf diffusive resistance, and leaf osmotic potential were measured every 1 to 2 d during the drying cycle and the day after rewatering. At completion of the experiment, five plants from each treatment were harvested for dry weight determinations.

Leaf area during the drying cycle was determined nondestructively by measuring the length and width of the center leaflet of each trifoliolate leaf. Area of the entire leaf was calculated from regression lines determined from at least 50 leaves of each cultivar grown under similar conditions.

Adaxial and abaxial leaf diffusive resistances were measured with a diffusion porometer (Delta Instruments) on the center leaflet of the youngest fully expanded leaf. For the common bean this was actually the first trifoliolate leaf. Because of the bushy habit of the tepary bean, a fully expanded, fully exposed leaf was selected. In each case, the leaf was immediately covered with a plastic bag, the petiole excised from the stem, and the water potential measured in a Scholander-type (17) pressure bomb (Soil Moisture Equipment). The leaf was then removed from the bomb and transferred to another zip lock plastic bag and stored on ice for measurement of osmotic potential. Preliminary experiments showed that the measurement of the water potential had no effect on subsequent osmotic potential measurements.

Leaves for osmotic potential determination were returned to the laboratory and stored at -20°C until measurement. The leaf

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<sup>2</sup> Abbreviations: WHR, white half runner; PPFD, photosynthetic photon flux density; DAP, days after planting.

<sup>3</sup> Turface is a product of IMC Chemical Group, Boston, MA.

osmotic potential was measured on extracted leaf sap with a vapor pressure osmometer (Wescor Instruments). Leaf sap was extracted with a tissue press which ruptures, filters, and deposits the leaf sap onto a filter paper disk designed to be placed directly in the osmometer (10). Triplicate measurements of leaf tissue with the same osmotic potential varied by no more than 0.5 bar (10).

Three d after rewatering, plants were harvested for dry weight determinations. Plants were divided into leaves, stems, and roots. Leaf area was measured with a leaf area meter (LI-COR model LI-3000). Plant parts were dried at 80°C in a forced convection oven for 48 h and were allowed to equilibrate with laboratory air for 2 h before dry weight was determined.

Pots were placed in alternating short rows on a greenhouse bench. Treatments and sampling sets were randomly assigned with each plant considered one experimental unit. Water potential, osmotic potential, and leaf diffusive resistance values are means of four replicates at each time point. Plant dry weights are the mean of five plants.

The experiment was repeated twice during the summer of 1982 and twice during the summer of 1983. Osmotic potentials were determined only during the 1983 experiments.

In a separate experiment, root growth was evaluated by growing plants in clear acrylic tubes 6.4 cm i.d. and 2.4 m in length. Tubes were filled with a 1:1 volume mixture of sand and surface, wrapped in aluminum foil, and placed at about a 60° angle in the greenhouse. Four tubes each containing a single plant were set up for each cultivar. The depth of the deepest observable root was measured every few days. Following 45 d of growth the root systems were removed from the tubes and stretched out along the greenhouse bench. Each root system was divided into 16-cm sections and the dry weight of each section determined. This experiment was repeated twice during the summer of 1983.

## RESULTS

The environmental conditions in the greenhouse varied among and within experiments. Although night temperatures were maintained at  $22 \pm 3^\circ\text{C}$ , the day temperatures ranged from 25 to  $34^\circ\text{C}$ . Soil temperatures also varied, with peak temperatures ranging from 24 to  $36^\circ\text{C}$ . The soil temperature of water-stressed plants was up to  $2^\circ\text{C}$  higher than that of the well-watered plants. This difference reached its maximum on the sunny days toward the end of the stress cycle. Photosynthetic photon flux density ranged between 850 and 2200, with  $1650 \mu\text{E} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$  as an average value. Although PPFD affected the temperature in the greenhouse and the rate at which the soil dried, levels never got

low enough to cause stomatal closure in control plants. RH ranged from 40 to 60% during the day and to around 80% at night.

**Growth.** Plant dry weight and leaf area were reduced by water stress in both species (Table I). Total dry weight of tepary bean decreased significantly more than the total dry weight of the common bean cultivars. The decrease was observed in leaves, stems, and roots with most of the difference in cultivars coming from the effect on root dry weight. Considering all four experiments, the average root dry weight in tepary decreased 24% whereas the common bean cultivars decreased 9% (WHR) and 7.2% (Pinto). Root:shoot ratios increased to the same extent in both species. Leaf area was also significantly reduced by water stress in both tepary and common beans. When compared to well-watered controls, leaf area of tepary decreased as much if not more than the leaf area of the common bean cultivars (Table I).

Although tepary was affected more by water stress than were the common bean cultivars, tepary produced more total dry matter and leaf area under both well-watered and water-stressed conditions.

Nondestructive measure of leaf area indicated that at the time of withholding water, 16 DAP, both species had the same leaf area (Fig. 1) and total dry weight (data not presented). Well-watered tepary and well-watered common bean had the same rate of leaf area expansion up to 22 d after planting at which time the rate of expansion increased, resulting in a larger leaf area in tepary than in common bean through the remainder of the experiment. This increase in leaf area was associated with a rapid increase in leaf number in tepary and not with an increase in the rate of individual leaf expansion.

Withholding water did not affect the leaf area of common bean until 7 d after the beginning of the treatment (Fig. 1). At this time, however, the rate of expansion decreased sharply and did not recover by 3 d after rewatering. Tepary responded differently to the lack of water. Leaf area expansion of the stressed plants decreased compared to the well-watered plants, with the rate of expansion slowly declining as the stress progressed. Tepary had a greater expansion rate than common bean at the time of rewatering, a difference which was maintained after rewatering.

**Water Relations.** Leaf diffusive resistance, water potential, and osmotic potential were similar in the two cultivars of common bean and were therefore combined for presentation. The water potential of well-watered tepary was usually 2 bars higher than the water potential of well-watered common bean plants (Fig. 2). This result was repeated in all experiments and was not

Table I. Effect of Water Stress on the Plant Dry Weight

Leaf area (LA), leaf dry weight (LW), specific leaf weight (SLW), stem dry weight (SW), root dry weight (RW), total plant dry weight (TW), and root/shoot ratio (R/S) for control (C) and water-stressed (WS) plants from one typical experiment. Means  $\pm$  SE, and per cent change for one typical experiment are presented.  $n = 5$ .

Plant	Treatment	LA	LW	SLW	SW	RW	TW	R/S
		$\text{cm}^2$	$\text{g}$	$\text{g cm}^{-2}$		$\text{g}$		ratio
Tepary	C	$386 \pm 63$	$1.3 \pm 0.18$	$3.42 \pm 0.11$	$0.44 \pm 0.1$	$0.86 \pm 0.22$	$2.6 \pm 0.5$	$0.47 \pm 0.06$
	WS	$157.6 \pm 21$	$0.6 \pm 0.08$	$3.75 \pm 0.13$	$0.17 \pm 0.03$	$0.58 \pm 0.07$	$1.34 \pm 0.2$	$0.76 \pm 0.06$
	% Change	-60	-54	9	-61	-32.6	-48	38
White half runner	C	$220.1 \pm 37$	$0.70 \pm 0.07$	$3.01 \pm 0.22$	$0.20 \pm 0.02$	$0.46 \pm 0.05$	$1.36 \pm 0.13$	$0.51 \pm 0.03$
	WS	$121.9 \pm 0.19$	$0.39 \pm 0.07$	$2.81 \pm 0.50$	$0.11 \pm 0.01$	$0.34 \pm 0.02$	$0.89 \pm 0.07$	$0.65 \pm 0.06$
	% Change	-45	-44	6	-45	-26	-35	21
Pinto	C	$150 \pm 11.9$	$0.50 \pm 0.03$	$3.37 \pm 0.08$	$0.20 \pm 0.02$	$0.44 \pm 0.02$	$1.14 \pm 0.06$	$0.63 \pm 0.04$
	WS	$81.2 \pm 8.1$	$0.31 \pm 0.01$	$3.66 \pm 0.14$	$0.13 \pm 0.01$	$0.43 \pm 0.01$	$0.86 \pm 0.04$	$0.90 \pm 0.07$
	% Change	-46	-38	9	-35	-2.3	-25	30

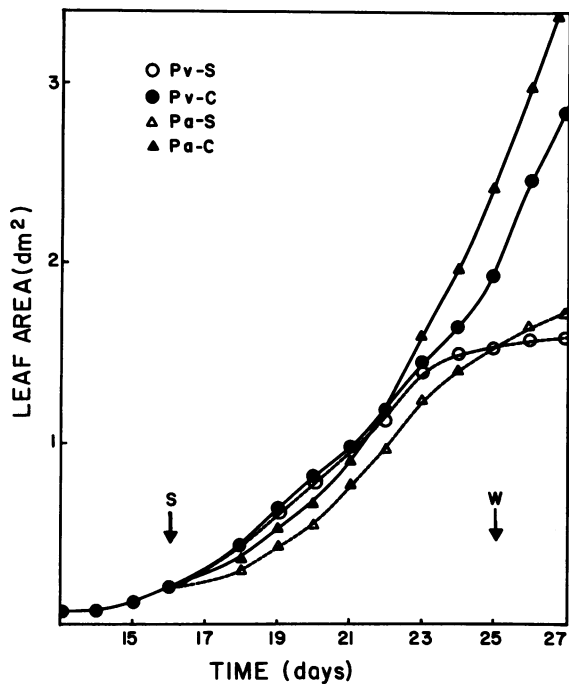


FIG. 1. Leaf area expansion for water-stressed and nonstressed common bean and tepary bean versus DAP. The results of the two common bean cultivars (WHR and pinto) were combined. Pv, common bean; Pa, tepary bean; (●, ▲), well-watered plants; (○, △), water withheld starting at day 16 and ending at day 25.

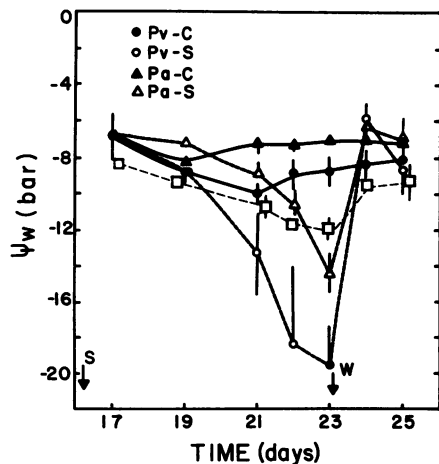


FIG. 2. Total water potential (○, ●, △, ▲) and osmotic potential (■) versus DAP. The results of the two common bean cultivars (WHR and pinto) were combined. Pv, common bean (○, ●); Pa, tepary bean (△, ▲); (●, ▲), well-watered plants; (○, △), water withheld starting at day 16 and ending at day 25. Because there was no significant difference between the osmotic potentials of the two species at any time, the data were combined.

associated with age or exposure of the leaf, or with plant age.

Water potential of the stressed common bean plants decreased rapidly from control levels of  $-7$  to  $-9$  bars to  $-18$  bars usually within 7 d after the beginning of treatment. The decrease in water potential of the tepary bean was much slower with a water potential of  $-14$  bars after 7 d of stress (Fig. 2). By 1 d after rewatering, the water potentials of both species recovered to values which in three of the four experiments were higher than control plants. Values returned to control values by the 2nd d after rewatering.

Osmotic potential of both common bean and tepary were about  $-9$  bars in the well-watered plants through the entire course of the experiment. Water stress caused a steady reduction in leaf osmotic potential during the drying cycle, reaching a minimum value of  $-12$  bars just prior to rewatering. There were no differences between tepary and common bean at any stage of the drying or recovery cycle (Fig. 2).

Stomatal resistance of both tepary and common bean increased 5 d after the beginning of the drying cycle with resistances of the upper surface increasing faster and to a greater extent than the lower surface (Fig. 3). Stomatal resistances in tepary increased earlier and to a greater extent than in common bean cultivars. Both species recovered to control values 2 d after rewatering.

There was a dramatic difference in stomatal sensitivity to water potential between the common bean and the tepary bean (Fig. 4). Whereas the stomates of tepary closed completely between  $-8$  and  $-10$  bars, the stomates of the common bean did not close appreciably until water potentials between  $-13$  and  $-18$  bars were reached.

**Root Growth.** Because rooting depth has been suggested as an important adaptation to dry environments, the depth of the deepest root was monitored nondestructively (Fig. 5). Both *P. vulgaris* cultivars behaved similarly with an average rate of root penetration of  $0.92 \text{ cm d}^{-1}$  from 10 to 60 d after planting. Tepary root systems, even at the time of the first measurement, 8 DAP,

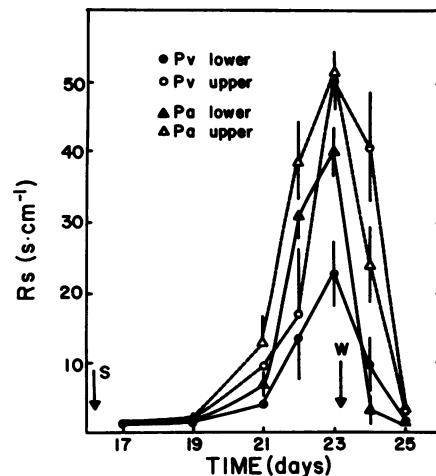


FIG. 3. Abaxial and adaxial leaf stomatal resistance for stressed common bean (Pv) and the tepary bean (Pa). (○, △), abaxial resistances; (●, ▲), adaxial resistances.

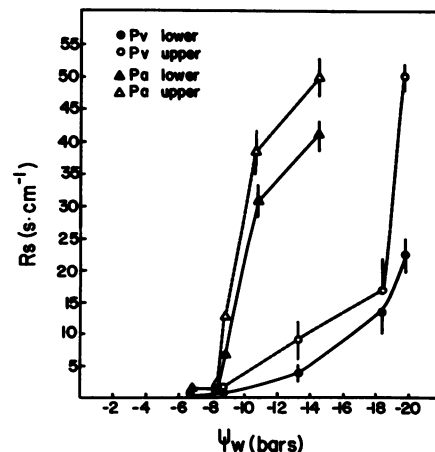


FIG. 4. Adaxial and abaxial stomatal resistance versus leaf water potential for water-stressed common bean (Pv) and tepary bean (Pa).

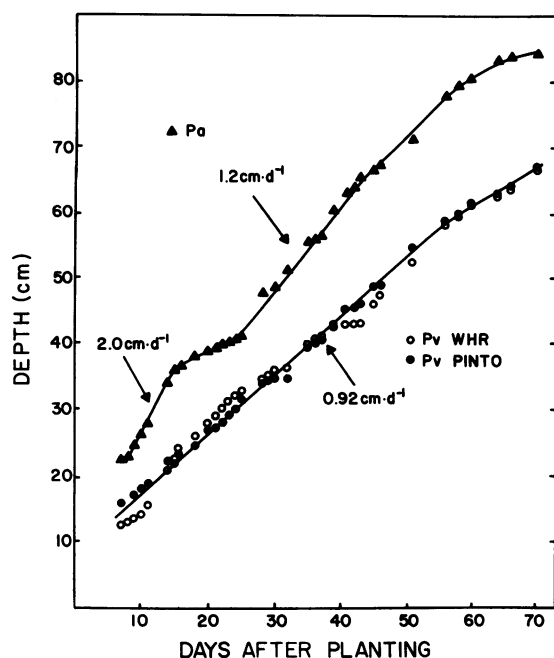


FIG. 5. Depth of deepest root penetration for common bean (Pv) cultivars WHR and pinto and the tepary bean (Pa). See text for details of experiment.

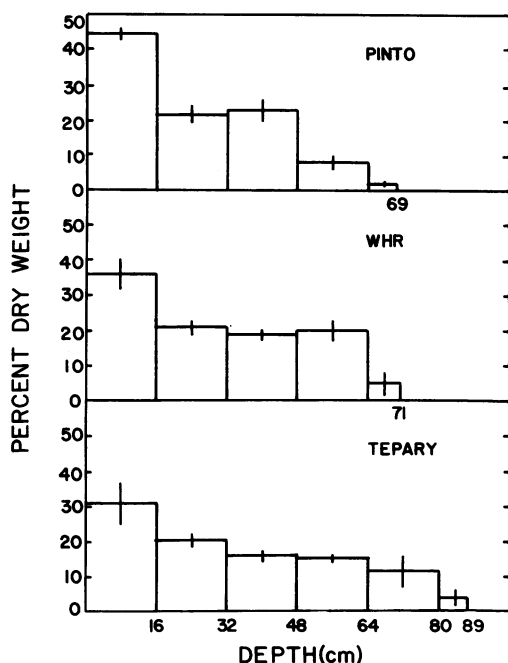


FIG. 6. Percentage of total root dry weight contained in 16-cm long sections of the soil column.

were deeper and continued to elongate faster, averaging  $2.0 \text{ cm d}^{-1}$  during the first 20 d of growth and  $1.2 \text{ cm d}^{-1}$  from day 30 to day 50. The deeply penetrating root system of the tepary bean resulted in a greater percentage of the root dry weight in the deeper layers of soil (Fig. 6).

## DISCUSSION

Tepary beans regularly outyield common beans under water limiting conditions (3, 15, 20). It was therefore surprising that, in these short-term experiments, growth of tepary bean was

inhibited as much as, if not more than, growth in common beans during the drying cycle (Table I). Although tepary leaf area expansion rate decreased earlier in the drying cycle than did that of common bean, the rate exceeded that of common bean toward the end of the stress period and appeared to recover faster after rewatering (Fig. 1). Economic yield, however, is measured as harvestable seed, not vegetative dry weight. Tepary bean produces extensive vegetative growth under well-irrigated conditions, but produces maximum seed yield with limited water supply (15). Nevertheless, substantial vegetative growth is a necessary prerequisite for maximum seed yield. The ability of tepary bean to cope with reduced water supply during vegetative growth is important in determining final seed yield.

The results from these experiments provide important insight into the mechanisms by which tepary copes with water stress. Leaf water potential remained higher in tepary than in the common bean during the entire stress cycle (Fig. 2). This is in part due to sensitive stomata. Whereas the stomata of the common bean did not begin to close until  $-13$  bars and were still significantly open at  $-18$  bars, the tepary stomata closed between  $-8$  and  $-9$  bars with almost complete closure by  $-10$  bars (Fig. 4).

Consistent with earlier observations on the common bean, adaxial stomata closed at a higher water potential than abaxial stomata (7). The results presented here extend this differential response of adaxial and abaxial stomata to the tepary bean.

Water potentials of well-watered tepary were usually 1 to 3 bars higher than those of well-watered common bean. Since the stomatal resistance was the same in well-watered common and tepary bean and the leaf areas did not differ until late in the experiment, the difference in leaf water potential was likely due to differences in root system characteristics.

Since decreased leaf water potential results from transpiration exceeding absorption, a larger root system, or a root system that more effectively occupies the soil, may delay the development of water stress. Water stress resulted in a more or less equivalent increase in the root:shoot ratio in both tepary and common bean. Experiments measuring the deepest penetrating root, however, suggest that tepary is more effective at exploring greater depths and perhaps greater volumes of soil. The difference between tepary and common bean is not in the total mass of root systems produced but in the distribution and possibly the efficacy of root systems to absorb water. Qualitative observations indicate that tepary roots are thinner and more highly branched than the common bean. Some preliminary microscopic observations revealed a thinner cortex region in tepary than in roots of common bean. Additional experiments are in progress to examine this point in more detail.

Depth of root penetration has been suggested as an important adaptation of tepary to water stress (18, 20, 21). Because common bean is a shallow-rooted crop (4), there is considerable interest in trying to move the character of deep rootedness from tepary bean into the common bean. Recent work is extremely promising. Common bean backcross lines derived from interspecific hybrids had roots as long as the tepary parent and significantly longer than the common bean parent (20).

Turgor maintenance through osmotic adjustment has been reported as an important acclimation response to water stress, enabling leaf expansion to continue and stomata to remain open at reduced water potentials (22). The role of osmotic adjustment in beans is unresolved. Changes in the osmotic potential of common bean leaves have been described in experiments where the osmotic potential of the rooting medium was decreased (5, 6, 11). It is unclear, if the observed decreases were, in all cases, true osmotic adjustment or a concentration of the cell sap due to tissue dehydration. Whether or not there is osmotic adjustment in response to soil drying is also unclear. Parsons and Howe

(14) reported the presence of osmotic adjustment in common and tepary bean; however, it is unclear whether the tissue was rehydrated prior to measurement. Kim and Stadelmann (8) observed no osmotic adjustment in the primary or first trifoliate leaves of common bean during a 6-d drying cycle.

The osmotic potential declined in both the common bean and the tepary bean from  $-9.0$  to  $-11.0$  bars during the course of the experiments (Fig. 2), but this decrease could be accounted for by tissue dehydration. This is supported by the rapid return to control levels the day after rewatering (Fig. 2). Apparently osmotic adjustment plays little role in the acclimation of either common or tepary bean to water stress.

Tepary bean is a dehydration postponer. Sensitive stomates closing at relatively high water potentials coupled with a deep extensive root system conserve tissue hydration. Although the sensitive stomates also result in an inhibition of growth, the integrity of the plant is maintained and the plant can make rapid use of water when it becomes available. With the recent success of interspecific crosses between *P. vulgaris* and *P. acutifolius* (1, 16, 19, 20, 21), the stage is set for attempts to move these specific characters into the common bean as a means of increasing its drought tolerance.

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